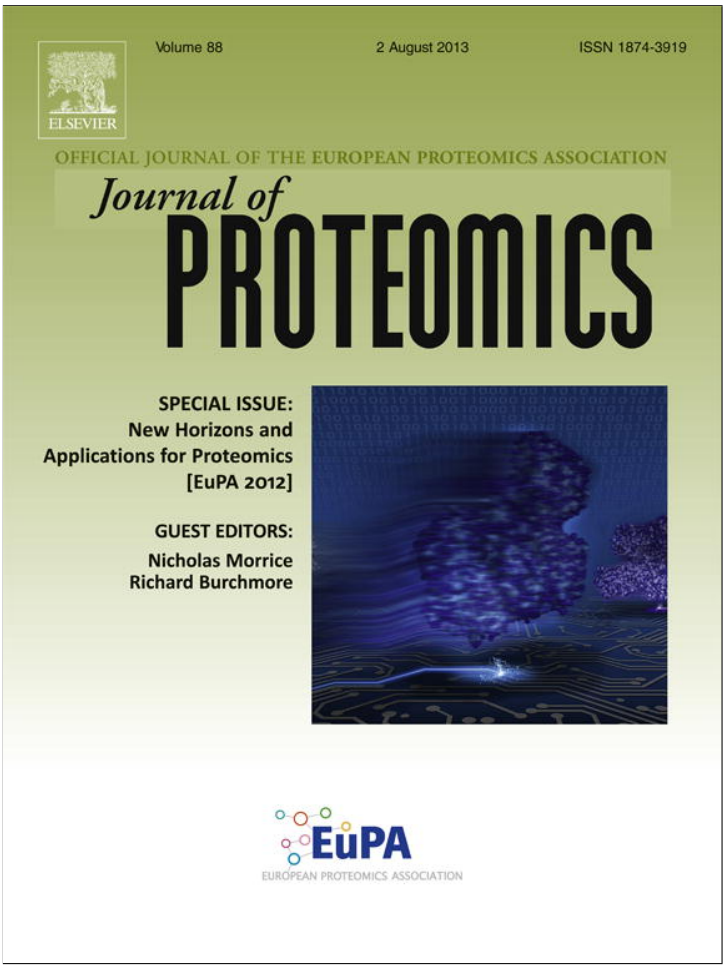

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Review

Muscle and meat: New horizons and applications for proteomics on a farm to fork perspective☆

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ABSTRACT

Meat consumption is an important part of human diet with strong implications in health, economy and culture worldwide. Meat is a proteinaceous product and therefore proteomics holds a considerable value to the study of the protein events underlying meat production and processing. In this article we will review this subject in an integrated "farm to fork" perspective, i.e. focusing on all the major levels of the meat producing chain: farm, abattoir and transformation industry. We will focus on the use, importance and applications of proteomics, providing clear examples of the most relevant studies in the field. A special attention will be given to meat production, as well as quality control. In the latter, a particular emphasis will be given to microbial safety and the detection of frauds.
This article is part of a Special Issue entitled: New Horizons and Applications for Proteomics [EuPA 2012].

Contents

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1. Introduction

Meat consumption is an important part of human culture since the dawn of ages and the formation of early civilizations. Indeed, in several cultures from the *Inuit* in Greenland to the *Sami* of Lapland, as well as the several peoples in the Mediterranean basin or the great plains of North and South America, meat production and consumption are key aspects defining and influencing not only local economy, but also its culture and ultimately its very essence. Meat production involves numerous domestic species, with several degrees of popularity, depending not only on cultural and religious beliefs but also on practical reasons and availability. The most popular species in the industrialized world, not including fish and shellfish, comprise cattle (*Bos taurus*, *Bos indicus* and hybrids), pig (*Sus scrofa*), sheep (*Ovis aries*), goat (*Capra hircus*), rabbit (*Oryctolagus cuniculus*), chicken (*Gallus gallus*), mallards and ducks (respectively *Anas platyrhynchos* and *Cairina moschata*, as well as their hybrids), the turkey (*Meleagris gallopavo*) and the Japanese quail (*Coturnix japonica*). Less familiar species are nevertheless particularly important outside of the Western world. These include the water buffalo (*Bubalus bubalis*), the dromedary (*Camelus dromedarius*), reindeer (*Rangifer tarandus*), Guinea-pigs (*Cavia porcellus*), geese (*Anser anser*), as well as ostriches (*Stuthio camelus*) and other ratites. Generally speaking production methods also vary considerably with species farmed, the location of the farm, availability of resources, etc. In the Western world, meat production systems are usually divided in two types: intensive and extensive, often with a third type in between, semi-intensive; or alternatively in commercial and subsistence farming. On a broad perspective, intensive systems include most of the pig, poultry and dairy production systems whereas grazing cattle, sheep and goats are associated to extensive systems. Nevertheless such boundaries are not clearly defined and tend to change according to the perspective and above all with geographical location. Although the farm is a key component of the meat production chain, there are other agents with equally important roles: the transporter, the abattoir, the meat processing plant, retail, regulatory agencies and ultimately the final consumer. Both quality and safety of a meat product is dependent on events that take place at the level of at least one of such agents. Accordingly, the meat producing sector is more and more often viewed in a "farm to fork" perspective, i.e. a global integrated approach allowing a more efficient, traceable and safe control of the chain and the characteristics of the product. These aspects are particularly important as a consequence of the expected rise in demand for food products of animal origin that are expected to increase significantly in the future particularly in emerging economies [1].

Proteomics can be defined as the science that studies the proteome, i.e. the study of the proteins being expressed in a given cell, tissue or fluid, organ, system or population. The importance of proteomics in animal science has recently been described [2] and demonstrated in numerous areas of animal production such as dairy products [3], *foie gras* [4], aquaculture [5], wool [6], or the monitoring of pollutant effects using shellfish [7]. We have recently reviewed, from the proteomics angle, the major events involved in the transformation of muscle to meat in a multi-species approach [8]. Nevertheless, and as meat is essentially a proteinaceous product, proteomics has necessarily a relevant role in the study of all aspects related to the meat producing chain. In this article, we aim to place proteomics in the context of meat science and in a "farm to fork" perspective, as schematized in Fig. 1. Accordingly, we will thoroughly address all the major components of the meat producing chains. In a first section we will concentrate on the use of proteomics at the level of the live animals that will be used in meat production. We will focus this section on aspects related to breed and genotype differentiation and at levels of feeding and management and handling of the animals. On a second section we will address the importance of

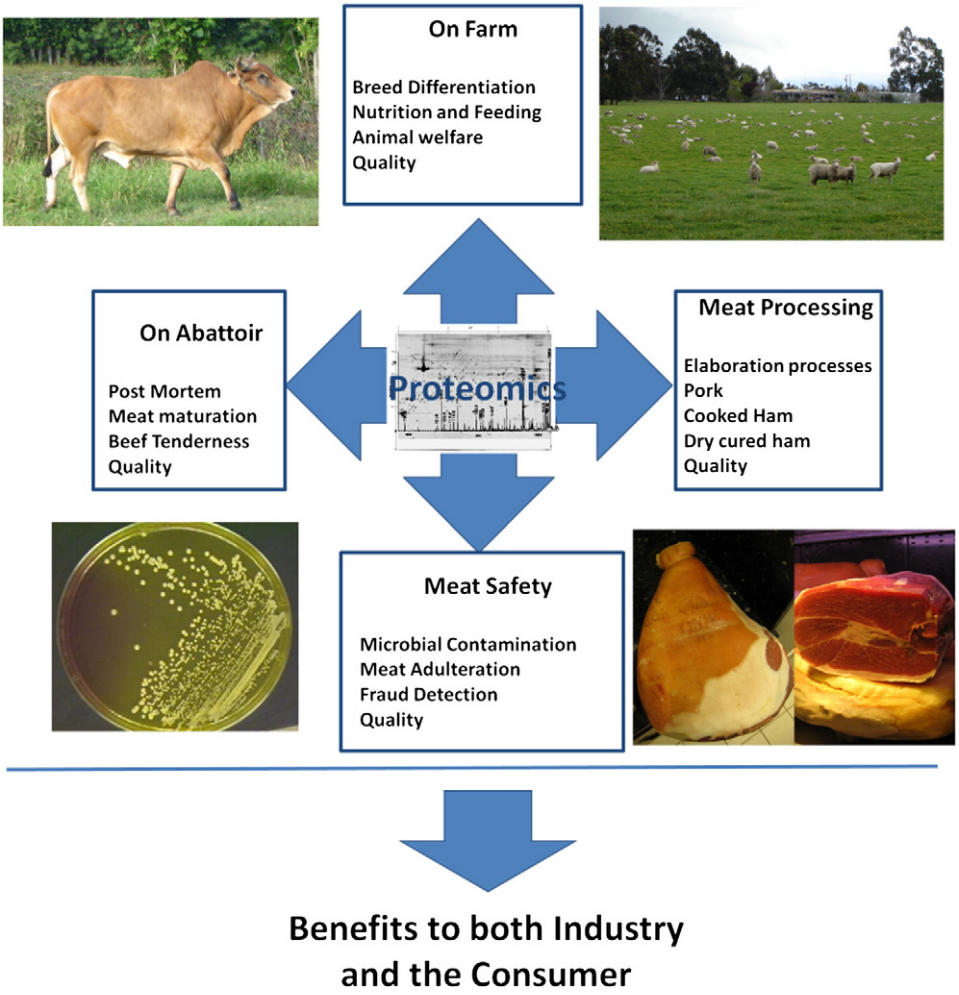


Fig. 1 – Schematic representation of the use of proteomics in meat science in a farm to fork perspective and including applications at the farm, abattoir, processing and safety.

proteomics in the slaughtering procedure. We will focus essential aspects related to tenderness in beef and the existence of PSE (Pale, Soft and Exudative) and DFD (Dry, Firm and Dark) meat in pork. In a third section we will address the use of proteomics in the elaboration of processed meat products, with a special focus on the production of dry-cured and cooked hams, an economically important delicatessen. A fourth section will be dedicated to the use of proteomics and peptidomics in the detection of meat frauds, particularly at the level of the introduction of cheaper meats in products labeled as being other meats. Finally, a last section concerns proteomics and microbial contamination of meat and meat products, a major consumer health hazard. All sections will be illustrated with concrete examples of applications, particularly concerning novel methodologies and approaches to the use of proteomics in meat production.

2. Proteomics and meat producing animals: on farm insights

The first step in any farm animal product making is necessarily the farm. Meat is no exception and the events that take place in the farm, i.e. the processes by which the animals are managed for later processing, will necessarily have major effects on the characteristics of the final product. There are numerous factors at the level of the farm with a significant impact on meat production; nevertheless, three factors are recognized as being the most significant: breed of the animal; feeding system and stress factors to which the animal is subjected to prior to slaughter. In this section we will illustrate how proteomics has been used to characterize changes at the muscle level on meat producing animal species in function of the above-mentioned three important factors.

The breed concept is relatively recent and in most cases farm animal breeds were defined and characterized from the 19th century onwards. Before that, animals were considered to be of a certain type that was defined by the local production characteristics and the purpose to which that animal was bred such as draught, meat or milk, just to name a few. Adaptation to particular environments, local pastures and production conditions may indeed be considered the key in breed formation; nevertheless, other factors have since been relevant, particularly the animal phenotype and appearance characters such as coat color, that in most cases are *sine qua non* conditions for an animal to be registered in a particular breed. From the 1960s

onwards, and particularly in the highly productive species such as pig and poultry, breeds have continuously lost importance in industrial production as commercial "hybrids" and strains designed for enhanced productivity became widespread in industrialized countries. Proteomics has been widely used in the study of differences between breeds and their effects on meat quality. In this section we will focus essentially on the differences between breeds that may be ascertained through proteomics at the level of the animal in a production environment. Nevertheless, in the next section we will address the use of proteomics in the context of the slaughtering procedure and the effects on meat quality.

2.1. *Breed differentiation—applications of proteomics*

Proteomics has extensively been used for breed differentiation in the pig. For a complete review on the subject, readers are advised to read Almeida and Bendixen [9]. Here we will focus on specific illustrative articles on this subject. Industrial pig breeds such as the Large White (LW), the Landrace (LR), Pietrain (PT) and Duroc (DC) are very different from local breeds from practically all regions of the world. Differences arise in almost all productivity aspects such as morphology and growth traits [10], reproductive traits [11], feed efficiency [12] or meat quality [13]. Such differences are also extended to protein expression levels, necessarily different between traditional local and industrial breeds. Accordingly, Xu et al. [14] studied the differences between the skeletal muscle proteome in LW and the Chinese Meishan breed, the latter being characterized for producing large litters and extreme fat deposition. According to the authors, differences may be found at the level of the expression of 25 proteins with a different role in metabolism, myofibrillar regulation, stress-related and other miscellaneous proteins. A similar study was conducted to compare the proteomes of the Longissimus lumborum muscle in LW and *Casertana*, a local Italian Mediterranean type breed, characterized for low growth and high fat deposition [15], using similarly to the previously mentioned example two-dimensional electrophoresis (2-DE) and protein identification via MALDI-MS, as well as a microarray transcriptomics approach. The authors identified protein differential expression at the level of the proteins involved in the glycolytic pathway in the Casertana breed whereas LW showed higher expression in cell cycle and skeletal muscle growth proteins and genes. Proteomics results have been recently used as a measure of traceability to detect not only breed differences between the Sire breed in pigs (in this case LW or DC), but also the rearing method and pig gender [16]. This interesting set of results point out to a major role of the proteins actin, myosin light chain, peroxiredoxin 6 and heat shock protein 73 in such differentiation. More recently and using a different approach based on Protein Chip Arrays, Mach and co workers [17] found four potential biomarkers of difference between the LR, Belgian LR, LW, DU and PT breeds at the level of the semi membranosus and longissimus muscles.

Breed differentiation has also been achieved using proteomics in other species, although to a lesser extent than in pigs. For instance, Zanetti et al. [18] conducted a study in the proteome of local Italian breeds chicken's pectoralis superficialis muscle using 2-DE and mass spectrometry. The authors defined a set of 11 proteins specific or differentially expressed, according to the breed studied. These include Cofilin-2, Myosin light chain 1 and Catenin binding protein. A similar approach was used by Almeida et al. [19] to study the effects of nutritional status in wild and domesticated (New Zealand White) rabbit gastrocnemius muscle. The authors noticed different expression profiles between the two breeds, particularly at the level of structural proteins like actin, myosin heavy chain or troponin, with higher expression levels in the domesticated breed.

Besides breed differences, proteomics has been extensively used to characterize proteome changes as a consequence of the expression of genes of particular relevance to meat science, leading to the existence of particularly important strains within a given breed. A well described example is the "double-muscle" or muscle hypertrophy phenotype. Double muscling is a phenotype associated to certain cattle breeds like the BBB (*Blanc Bleu Belge*) or the *Piedmontese* in which the animals with a mutation in the myostatin gene (also known as growth and differentiation factor 8) causes an increase in muscle mass, particularly in the regions of the fore and hind quarters [20]. Although the phenotype has been essentially described in cattle [20], it has also been described in sheep, particularly in Texel and Norwegian white [21], whereas a similar phenotype was described in fish [22]. Proteomics studies have compared the effect of the myostatin gene in heterozygote and homozygote young Belgian blue bulls at the level of the *semitendinosus* muscle [23]. Authors found a total of 28 differentially expressed proteins and were able to identify 13 as a consequence of myostatin gene deletion, particularly structural proteins like myosin binding or myosin regulatory light chain, as well as others like phosphoglucomutase and sarcosin. The authors finally relate changes in protein expression with the increased muscle phenotype and suggest that myostatin negatively controls the number of fast-twitch glycolytic fibers. A similar approach was later conducted by the same research group to study muscle hypertrophy in Texel sheep [24] at the level of several skeletal muscles. Authors found major differences at the level of glycolysis proteins, as well as glutathione S-transferase, several heat shock proteins and transferrin.

2.2. *Farm animal feeding—nutritional status influences muscle proteome*

Feeding accounts for a variable but necessarily high proportion of the inputs in every animal production system. These are particularly high in intensive productive systems such as pig and poultry [25] or in systems based in natural pastures but with huge feed gaps like Mediterranean and Tropical systems that require the use of supplementation [26,27]. Additionally, feeding strategy strongly conditions not only the animal production standards but the very quality of the meat product, including the protein composition. Accordingly, proteomics has been used to address this issue. An interesting example is a study by Shibata et al. [28] in which the skeletal muscle proteome of Japanese Black cattle was compared in animals fed with grass and with grain. A total of nine proteins were found to have differential expression. Similarly to breed differences, differences arising from the comparison were obtained essentially on structural proteins like troponin, tropomyosin or myosin light chains. The authors later validated the results using Western Blot for Myosin

Heavy Chain, Troponin T and Troponin I. Results obtained confirmed higher expression levels for the three proteins in grazing cattle (slow type), whereas the opposite was recorded for the fast type. A similar study was used to compare the Longissimus dorsi muscle profiles of Korean *Hanwoo* steers at different stages of the fattening process, respectively 12 and 27 months of age in a study the focused essentially age variations with implications in feed management [29]. In this study that used 2-DE, the authors found eight proteins with differential expression profiles, including zinc finger 323, myosin light chain (higher in the older animals), triosephosphate isomerase and succinate dehydrogenase that had higher expression levels in the younger animals, relating these results with fat content and finishing stage. A similar approach was conducted in the Thai chicken breed [30] where the pectoralis muscle proteome was compared at different phases of the growing period (0, 3, 6 and 18 weeks of age). Five proteins were associated to chicken age: phosphoglycerate mutase, apolipoprotein A1, triosephosphate isomerase 1, heat shock protein 25 kDa and fatty acid binding protein 3 indicating important differences in energy metabolism as a consequence of the ageing process.

2.3. *Proteomics and farm animal welfare indicators*

Another process taking place on the farm and that have an influence in the meat quality is the way animals are raised and transported to the abattoir and their relation to the stress imposed upon the animals. In fact, these are highly critical steps that have additional important implications in animal welfare and consequently the public perception of the meat producing chain. The use of proteomics to address this issue is still in its infancy. To the best of our knowledge, only one example was found in the literature where this issue was specifically addressed in the muscle tissue of farm animals. Such an example is the experiment by Hazard et al. [31] on the effects of restraint and transport in the muscle proteome of chickens. These authors applied a 2 h restraint to chickens that were later euthanized and the *tensor fascia latae* and *biceps femoris* muscles used in the proteomics analysis. A total of 29 proteins were found to have differential expression, 37% of which with a function in glycolysis and 14% in cell structure. This analysis was conducted in parallel with both transcriptomics and metabolomics approaches and seem to indicate that the restraint period resulted in a repression of glycogenolysis and glycolysis in the thigh muscle of chicken. Albeit the relatively lack of examples, the demonstration of proteomics in the detection of biomarkers of stress response has been described for other tissues/fluids and farm animals such as blood serum in two cattle breeds reared under different production systems [32] or in swine subjected to different stocking densities [33]. It would therefore be interesting to conduct a study at the muscle level in the animals used in the two above-mentioned experiments.

2.4. *On farm insights: concluding remarks*

From all the previously referred studies, it is clear that proteomics has been extensively used at the muscle level to differentiate breeds and strains in several species: cattle, pig, sheep, chicken and rabbit, as well as for studies related to feeding strategies and stress induced by transport and different management systems. Interestingly, most of these studies used a traditional approach based on 2-DE and protein identification using Mass Spectrometry and particularly MALDI, probably the most common initial approach and readily accessible technology in every proteomics experiment. Although interesting and, up to a certain level, rather complete, 2-DE based approaches have a major setback that the number of proteins discernible using the approach is very limited. Nevertheless, with a wider access of animal and meat scientists to proteomics and mass spectrometry platforms, it is likely that novel technologies such as the high-throughput iTRAQ (Isobaric tag for relative and absolute quantitation [34] based technology, allowing the full characterization of entire proteomes as used by Hornhoj et al. [35] for the swine muscle, will be more and more frequently used to address these issues, with important applications in production system characterization, traceability and the quality of the meat products. Nevertheless, it is relevant to note that every proteomics approach is severely limited by the number of protein entries existing in public databases. Generally speaking these are higher for sequenced organisms such as cattle (over 180,000 entries in the NCBI database) and very well studied organisms such as pig (95,000 entries) or the chicken (62,000 entries), but low in many of the others (e.g. 3600 for the goat or 1400 for the duck). This implies that proteomics studies may not be fully successful when using farm animals poorly represented in the databases and depending to a large extent on the existence of homologies with other species [36]. It is therefore of utmost importance to increase the number of entries for farm animals in public databases and particularly in proteins of the muscle.

3. Proteomics and post mortem storage: implications for meat quality

3.1. *Proteomics characterization of the postmortem process*

Proteomics is a powerful technology to study global changes of proteins occurring during postmortem storage. This has shed new light on degradation of proteins like actin and myosin heavy chain thought to be unaltered during postmortem storage by the presence of degradation products in post mortem samples [37]. By comparing the postmortem changes in protein composition between the soluble and insoluble protein fractions, we were able to look at the changes in solubility during postmortem storage [38]. This study indicates a connection between the stability of myofibrillar proteins and the solubility of easily soluble proteins, such as metabolic enzymes and cellular defense/stress proteins. The occurrence of these easily soluble proteins in the insoluble protein fraction could be due to precipitation or aggregation, thereby going from a soluble to an insoluble state. Different mechanisms might be responsible for this change in the protein solubility, e.g., isoelectric precipitation caused by the pH decline or modification of proteins.

A clear shift in energy metabolism in the muscle post mortem compared to the living animal has been observed, with an increase in enzymes involved in both the glycolytic pathway as well as in the TCA cycle [39]. These findings

suggest that an increased aerobic energy metabolism occurs the first hour after slaughter. The increased aerobic energy metabolism will probably affect the rate of glycolysis in muscles after slaughter and eventually lead to variation of meat quality. During the next 24 h of post mortem storage proteins in the biochemical network cooperating to prevent muscle cells from reducing the ATP level, cellular stress responses and cell death were affected [40]. The results show that all identified metabolic enzymes are either involved in enzymatic reactions of the glycolytic and TCA pathways or associated with energy production. This indicates that the level of ATP is maintained for several hours after slaughter, and the energy production is still operative under the conversion of aerobic metabolism to anaerobic metabolism in muscle.

Muscle cells are under stressful conditions after slaughter caused by nutrient and oxygen depletion. This is supported by the finding of stress and defense proteins, which were changed in abundance early postmortem. The protective functions of these proteins are probably to delay cell death, thus diminishing the impairment of stress. These changes could reflect important mechanisms related to development of a satisfactory meat quality. Several studies have shown a decreased abundance of heat shock proteins 27 and 70, both known to inhibit apoptotic pathways, suggesting an increase in apoptosis in the muscle during tenderization [38,41].

Electrical stimulation (ES) of carcasses shortly after slaughter accelerates glycolysis resulting in a rapid pH decline and earlier onset of rigor mortis, and is frequently used to improve tenderness [42,43]. Proteome analyses of electrically stimulated muscle show an earlier degradation of metabolic enzymes and creatine kinase supporting higher energy consumption and depletion of ATP [44,45]. In the same studies a faster rate of proteolysis is suggested by increased activity of proteolytic enzymes, decreased abundance of troponin T, desmin and actin and a higher abundance of HspB1 and α-crystallin. Myofibrillar protein instability also indicates an earlier initiation of stress responses due to ES.

3.2. *Protein markers for beef tenderness*

Tenderness is considered the most important quality trait of beef by consumers followed by juiciness and flavor [46]. A large variation in beef tenderness has been reported, and identification of markers for meat tenderness in cattle has gained attention in recent years. Quality traits of muscle foods are influenced by a number of different factors such as genetics, environmental factors and processing conditions. Thus understanding the variations and different components of the proteome with regard to certain quality or processing parameters will lead to knowledge that can be used in optimizing the conversion of muscles to meat [47–49]. There are three main factors that determine meat tenderness [50]. These are the background toughness, the toughening phase and the tenderization phase, with the two latter phases taking place during the post mortem storage period (reviewed in [51]). The toughening phase is related to sarcomere shortening caused by muscle contraction during rigor development, while the tenderization phase is the result of degradation of structural proteins [52]. Given that the protein composition of raw materials may be variable it is important to be able to understand the factors that are involved in the development of tenderness.

Proteomics has also been used by several groups to identify potential protein markers for tenderness in beef [41,53–62]. The power of these candidate markers to explain variation remains unclear, but it is certain that they will contribute to building a better picture of this complex process. Proteins suggested to play a role in tenderness are involved in glycolysis and energy metabolism, heat shock proteins, oxidative stress resistance, myofibril structure and proteolysis. Proteolytic products from mitochondrial membranes were more abundant in tender muscle compared to tough muscle analyzed shortly after slaughter [56]. This indicates an increased disruption of the mitochondrial membranes in the tender muscle samples which can be related to caspase activation.

A major challenge is to extract relevant information from all these studies and identify the cellular pathways that are involved in tenderness development. As an effort to do a functional analysis of beef tenderness proteins, Guillemin and co-workers analyzed the relationship and interaction between 24 published potential markers of tenderness [63]. This study pointed out apoptosis, HSP functions and oxidative stress resistance as cellular pathways strongly involved in tenderness. Using this interaction pathway analyses it is also possible to study differences between muscles in the carcass as different meat cuts and muscles have a different protein profile and is also highly variable in tenderness.

So far, most studies of proteome changes related to meat quality have been done using 2-DE for separation of proteins. In a recent study we compared results from isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and 2-DE analysis [62]. A number of the proteins which have previously been related to tenderness were found to change in abundance between tender and tough samples, both in iTRAQ and 2-DE analysis. Even though the overlap in significantly changing proteins was relatively low between the iTRAQ and 2-DE analyses, certain proteins predicted to have similar function were found in both analyses and showed similar changes between the groups, like structural proteins and proteins related to apoptosis and energy metabolism. In a conclusion, most of the proteins found to change significantly between tender and tough sample groups (both in iTRAQ and 2-DE analysis) are in line with previous reports on meat tenderness. The limited number of proteins detected by both analyses can be explained by the fact that the methods are based on different principles for identification and quantification.

The fact that different results are observed in the various studies performed to unleash potential markers for tenderness reflects the complexity of this trait. Many factors may influence on tenderness development in muscle and meat, thus a protein marker in one animal may not be valid in another animal due to different breeds, gender, treatments or other unknown factors. However, some general mechanisms seems to be involved including glycolysis and heat-shock proteins as well as some other candidates that should be further investigated and validated.

Comparing proteomics investigations with metabolomics data from the same set of samples will provide more information on the activity and relevance of the proteins

that appear to have a changed abundance according to tenderness. This has been done in two studies of beef from Maremma and Chianina cattle [59], and provides useful information not only between the proteins and metabolites but also to differences in metabolism between the two breeds. By comparing the two breeds it was suggested that the metabolic rate post mortem is breed specific. This directly influences the post mortem time necessary to obtain tender meat and demonstrate a practical link between industrial needs and -omics tools.

3.3. *Proteomics, water-holding phenotypes and impact on pork quality*

Inadequate water-holding capacity (WHC) is a major issue in pork quality, which may lead to dark, firm and dry (DFD) meat with enhanced water binding capacity or pale, soft and exudative (PSE) meat with poor WHC. PSE meat may be the result of genetics, stress at slaughter and the rate and extent of post mortem pH decline (reviewed by [64]).

The halothane gene is considered to be a genetic cause of PSE pork [65]. Proteome analyses of pigs of different HAL genotypes demonstrated several differences in the sarcoplasmic protein fraction [66]. In the nn homozygous mutants a higher abundance of glycolytic enzymes were observed together with lower abundance of enzymes involved in aerobic ATP synthesis and heat shock proteins.

In a recent study, Di Luca and co-workers identified proteins in centrifugal drip from pork with varying WHC phenotypes [67]. They identified many proteins changing in abundance during post mortem storage of the meat, but only HSP70 were changed in abundance according to WHC. It was more abundant in the DFD and low-drip group compared to the PSE and high-drip group of samples. This may indicate a protective effect towards protein denaturation and aggregation in DFD and low-drip pork or a different cellular localization between the groups.

3.4. *Proteomics and the slaughtering process—concluding remarks*

Proteomics has added new knowledge to the understanding of post mortem metabolism and tenderization in meat. In addition studies of the molecular events occurring during electrical stimulation and pre-slaughter handling of animals has lead to a better understanding of these factors. Several potential protein markers of tenderness and meat quality traits has been suggested, however, the impact of the individual markers are just partly understood. Meat quality is a complex trait involving many biochemical pathways, proteolytic proteins and cell death. Thus it is probably not possible to find one marker for meat quality, but rather an orchestra of different proteins that together may explain high quality meat.

The regulation of post mortem tenderization on the molecular level needs to be further investigated to improve the understanding of such mechanisms. Furthermore there is a need for better prediction of meat quality through molecular markers to improve meat quality. In such a context, proteomics will undoubtedly play a major role.

4. Proteomics and processed meat product technology: an emphasis on hams

Meat is used worldwide as a source of proteins with quantities that presently vary from 31 g per day in Africa to 224 g per day in developed countries [68]. In the last 50 years the amount of pork meat that is consumed accounts for almost the same quantity of the sum of beef, chicken and lamb meat [69]. In the USA about 22 kg of pork meat per capita per year are consumed constantly in the last 30 years whereas beef meat consumption is decreasing from 32 to 27 kg per capita and broiler meat is increased from about 14 to about 27 kg per capita [70]. In China the consumption of meat is 71 tons, of which 75% is pork meat. A significant part of the consumed pork meat is represented by processed meat, i.e. mainly dry cured ham and cooked ham. The latter represents about 26% of delicatessen food products sold in Europe, with France, Spain and Italy being top consumers. In Italy, in 2010, the amount of cooked ham and dry cured ham consumed per capita was 4.7 and 4.2 kg, the difference being likely due to the 3–4 fold higher price for dry cured ham [71].

Overall, the advantage of processed pork meat with respect to fresh meat is the long shelf life that varies from months to year, especially for the dry cured ham. This was the main reason why in the pre-industrial period country people developed pork meat conservation processes. These processes are based on the use of salt as preserving agent, thus accounting for the relevance of salt in the pre-industrial economy.

Given the extensive consumption of processed pork meat in western countries diet, it is surprising that the technological processes for the production of dry cured ham and cooked ham have been scarcely investigated, with the latter even less investigated than the former [8].

4.1. *Production of dry cured ham—a proteomics perspective*

Dry cured ham is a food prepared in different countries. In Portugal the most important type of ham is *presunto*, a dry cured ham similar to Spanish *jamón* and Italian *prosciutto*. The most famous are *presunto* from Chaves and *presunto* from Alentejo, which is made from black Iberian pig. *Jamón* from Spain is either *jamón serrano* (meaning ham from the *sierra* or mountains) or *Jamón Ibérico* and is made from black Iberian pig. In Italy *prosciutto* is made in several regions, the most famous being produced in Parma (*Parma* ham) and in Friuli (*S. Daniele* ham). In France it is well known the *jambon* from Bayonne and in China the *Jinhua* ham, the most traditional meat product.

The production of dry cured ham is usually based on three phases. Phase time length, the amount and type of salt differ from country to country, from region to region, from local producer to local producer, leading to distinct, peculiar products with significantly different taste, texture, color and overall quality. Here, we report a standard Italian manufacturing process. In phase 1 pork skin is covered with dry salt whereas low-fat parts are covered with humidified salt. Meat is held at 1–3 °C, at high relative humidity (RH>80%) for two-five days. An exudate is formed during this phase. At the end of the period, meat is cleaned from salt, lightly re-salted and kept at 1–3 °C, high relative humidity

(RH > 80%) for other 15 days. An exudate is also formed during this step. In phase 2, legs are placed to rest for 60–80 days in air circulation at 2–4 °C and decreased relative humidity to enhance meat dehydration. About 8% of meat weight is lost during phase 2. In phase 3, hams are stored under controlled conditions of temperature (14–18 °C), air circulation and humidity, for 12 months and over. About 15% of meat weight is lost during phase 3. Overall, about 50% of meat weight is lost during the whole process. The technological conditions affect protein solubilization, proteolysis, lipolysis and generation of volatile molecules, generating typical texture and sensory characteristics, i.e. the quality of the final product.

Proteomic analyses that were carried out on meat to ham transformation were aimed at correlating protein pattern of either meat or exudates with ham quality. Because quality is strongly dependent on the properties of the starting meat, a proteomic investigation [72] characterized the differences in protein pattern associated to polymorphisms of PRKAG3 and CAST genes in *biceps femoris*. The former gene encodes for the 5′-AMP-activated protein kinase subunit gamma-3, protein involved in the regulation of energy metabolism, whereas the latter gene encodes for calpastatin, an inhibitor of the protease calpains. The PRKAG3 genotype correlates with a modification in intensity of 40 spots. Thirteen spots were identified by peptide mass fingerprinting, of which 11 were related to enzymes involved in energy metabolism (muscle creatine kinase, enolase 3). In the CAST genotype, 13 spots showed statistical relevant differences in intensity. Three exhibited a higher concentration in CAST1 genotype while the other ten exhibited a higher concentration in CAST2 genotype. This investigation also showed that the degree of salt strongly affects proteolytic activity. Furthermore, the abnormal glycogen contents of PRKAG3 pig muscle affects the post-mortem metabolic potentials and muscle pH decline. Moreover, the modifications triggered in *biceps femoris* proteome by high and low salt level were investigated [72]. Forty-five spots differed in intensity depending on high and low salt content. Twenty-one were identified by mass spectrometry with nine being myofibrillar proteins of which three α-actin, one a fragment more abundant at low salt and two as whole molecules more abundant at high salt. Moreover, five spots were identified as various myosin chains, being more abundant at low salt. A fragment of the myosin heavy chain and of desmin was found to be more abundant at high salt. Salt also affects six spots identified as metabolic enzymes. These include lactate dehydrogenase A, bisphosphoglycerate mutase 2, fragment of mitochondrial creatine kinase, Va subunit of cytochrome *c*-oxidase and glyceraldehyde-3-phosphate dehydrogenase. A proteomic analysis was also carried out aimed at correlating protein pattern and pastiness, a mastication taste due to excessive softness, mushy texture and unpleasant flavors of dry cured ham [72]. Fifty-five proteins were found differently abundant at high and low pastiness. Among the twenty proteins that were identified, one was also influenced by *PRKAG3* genotype and six by salt level. Seven proteins were myofibrillar or cytoskeletal proteins, more abundant at low pastiness, being two of them α-actin, three α-actin fragments, two a fragment of desmin and a fragment of myosin heavy chain. Overall, it seems that pastiness is associated to the activity of proteolytic enzymes that, in turn, are affected by salt levels.

The effects of the ripening period on meat maturation were investigated by a 2-DE analysis [73]. The water soluble and the myofibrillar proteomes from raw meat and dry cured hams ripened for 6, 10 and 14 months were analyzed. The ripening phase was characterized by intensive protein degradation due to the action of several endogenous enzymes. In the sarcoplasmic fraction a reduction in intensity of different spots was detected. Particularly, it was found the disappearance of creatine kinase and major modifications in spots related to 3-phosphoglyceraldehyde dehydrogenase and enolase B. It was also found the appearance of four spots, two with acidic pI, identified as tropomyosin alpha and beta chain with molecular weight of 38 kDa, and two with molecular weight of 14 kDa. The appearance of tropomyosin in the sarcoplasmic fraction was explained by an increased solubility in water due to salt effects and a minor susceptibility to proteolytic enzymes. In the myofibrillar component, at 6 months, the complete degradation of myosin light chain 3 and partially degradation of myosin heavy chain was detected. At 10 months other fragments of myosin heavy chain and the complete absence of tropomyosin spots were observed. Finally, at 14 months, the complete proteolysis of actin and myosin light chain was observed. It was also found that some spots were present only at intermediate time indicating that some proteins are released and further processed.

A proteomic study of the insoluble fraction of the *semimembranosus* and *biceps femoris* muscles from Bayonne ham was carried out [74]. The different muscle localization leads to a different salt content and proteolytic events. Indeed, the *semimembranosus*, which is at meat surface, exhibits higher levels of salt and a lower content of water than the *biceps femoris*. In the former muscle, several proteins involved in the energy metabolism were over-expressed, whereas in the latter, that is in the internal part of the meat, myofibrillar proteins were over-expressed. Moreover, in the *biceps femoris* a higher number of spots related to fragment proteins than in the *semimembranosus* was found.

Dry cured ham contains generally 2.5 g of salt per 100 g of product. This poses a serious health issue for persons with high blood pressure and may lead to a reduction in the amount of dry cured ham in their diet. In order to circumvent this issue, investigations are ongoing aimed at the production of high quality ham with as low as possible salt content [75,76]. A proteomic investigation analyzed the exudate formed during the first 18 days of dry cured ham processing (phases 1 and 2), with and without a pre-step in which a pressure was applied to the raw meat in order to obtain meat with standard shape and dimension. Exudate protein concentration remained constant between days 1 and 5 at a value of about 3 mg/ml, and significantly decreases at day 18. A 1-DE analysis showed a higher amount of two protein bands with low molecular weights at day 1 than at day 5 and 18. Interestingly, three bands showed a higher concentration at day 18 compared to days 1 and 5. A 2-DE analysis of proteomes at different time periods was carried out in the pH ranges 4–7 and 7–10 and protein identification is currently being undertaken.

4.2. *Production of cooked ham—a proteomics perspective*

Similarly to dry cured, cooked ham production consists of three phases. However, significant differences are present. In phase 1, pork meat undergoes a process where a brine containing salt at different concentrations, usually between 15 and 45%, and ingredients, such as casein or spices, is injected by the use of multiple syringes. In phase 2, 200–300 brine-treated meat pieces are tumbled in big containers for different time lengths, at distinct temperatures, in order to favor brine distribution within meat. This phase causes the breakage of muscle cell and the release of a protein exudate both within and outside the meat. This exudate is relevant for the final quality of the product because it acts as a glue conferring typical texture and cohesion to the cooked ham. In phase 3, different meat pieces from *Biceps femoris*, *Quadriceps femoris* and M. *semimembranosus* are assembled, packed in closed bags and cooked at 68–72 °C to reduce the microbial load. Upon refrigeration, the cooked ham is aged for at least one month in order to obtain the final product. During this period, proteolysis caused by remaining enzyme activity and protein renaturation take place conferring to the cooked ham typical texture qualities.

A proteomic investigation characterized the first phase of pork meat to cooked ham production [77]. The exudate formed during the brine injection into meat was analyzed by 2-DE and MALDI-TOF spectrometry. The first dimension was carried out with runs at pH 4–7 and 7–10 in order to improve the resolution. For the latter pH range, at 30% brine, the second dimension led to separate 34 proteins, accounting for 46% of protein content. By peptide mass fingerprint analysis creatine kinase M chain, fructose biphosphate aldolase A and beta enolase were identified. In the pH range of 4–7 2-DE analysis separated 41, 37 and 42 protein spots in exudates at 15%, 30% and 45% brine with nine common spots accounting for 90, 88 and 66% of the total averaged protein quantity, respectively. Additionally, caseinate was identified only at 45% brine, as this protein is injected during treatment to improve product quality and is reported in the ingredient list. Other identified proteins were albumin, tropomyosin alpha chain, tropomyosin beta chain, myosin light chain 1 and 2, alpha actin 1. Some of them were differentially abundant at different brine levels. Specifically, tropomyosin alpha chain was found to be more concentrated in exudates derived from meat injected with brine at 30%, myosin light chain 1 and myosin regulatory light chain 2 were more abundant with brines at 15% and 30% with respect to brine at 45%. Actin alpha 1 was extracted in quantity seven and four fold more from brine at 15% than 30% and 45%, respectively. Actin alpha 1 isoforms were differentially extracted at various brine injection levels. Also the comparison of the abundance of less populated proteins that were not identified, indicated a relative extractability of muscle proteins by different brine levels, leading to a fingerprint pattern as a function of brine level. A comparison was also carried out on the identity and quantity of proteins differentially extracted at 30% brine varying the temperature from 4 to 10 °C and the time length of tumbling from 4 to 20 h.

In another investigation, the amount of myofibrillar proteins extracted during tumbling at 7 °C was analyzed by 2-DE and found to reach a plateau at about 5 h [78]. The amount of extracted proteins was also determined during cooking between 35 and 75 °C. It was found that actin and desmin, an interfilamental constituent of myofibrils, were predominantly extracted at temperature below 58 °C. At higher temperatures the amount of extracted proteins sharply decreases with exception of albumin.

Different proteins and different quantities of proteins extracted from muscles significantly impact on the final texture of the meat product and, hence, on its quality. This information can complement the nowadays only experience-based decision on meat process conditions in the industrial production.

4.3. *Proteomics and meat processing—concluding remarks*

Meat processing, as associated to packaging, preservation and subsequent sale is one of the key issues in the meat processing chain, frequently the one that is more visible or perceptible to the final consumer, being the ultimate step in the farm to fork chain of events. Despite its overwhelming importance in the context of fundamentally proteinaceous product, as seen in the previous two chapters, proteomics has had a very limited use in the context of meat processing. One exception is the manufacture of dry cured and cooked hams, products in which proteomics has been applied with success, as seen in this section. Nevertheless, similar approaches could equally be conducted to study the processing of meat products such as sausages or hamburgers, with possible interest not only in the manufacturing context and technological properties, but also with implications for consumer health.

5. Meat authentication and fraud detection—a role for proteomics and peptidomics

In modern society, consumers exert an increasing demand claiming for detailed and reliable information about the food they consume. The reason is that each particular lifestyle, together with religion, diet or health issues, strongly influences consumer's choice on the foods they buy depending on their composition. To meet these criteria, clear and precise labelling is essential, especially in the case of processed food products where visual differentiation of constituents is difficult. An example of this is the current increasing demand for traditional and regional meat products, perceived by some people as higher-quality and healthier products. In such products a correct labelling and protection must guarantee their quality and authenticity [79]. Legal authorities must take charge of this task by means of specific regulations capable to protect against misdescription and fraud, which is generally carried out with the aim of increasing profit. In the case of meat foods, there is a legal requirement to indicate and quantify each animal species contained in each product separately [80]. Legal bodies must assure the performance of these requirements from food producers by means of sensitive, accurate and reliable methodologies in order to ensure that illegal or accidental practices do not arise. The main problem sources associated to fraudulent practices in the

meat industry deal with the origin of meats, substitutions of meat ingredients (by other animal species, tissues, fat and/or proteins), alterations in the processing methods and/or additions of non-meat components such as water or colorants [81].

5.1. *Analytical strategies and meat fraud—a role for proteomics and peptidomics*

Different analytical strategies have been developed up to date with the aim to assess meat authenticity and prevent fraud. Analysis of stable isotope ratios and trace elements has been used for example as a way to know the geographical origin of meats, as well as a way to know the methods that have been used to fed the animals. Ratios of elements such as $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ can change in the soil and drinking water of the different parts of the world, but also in the different feeds that can be used to grow the animals (grain vs pasture, for example). As these variations are then incorporated into animal tissues, analyses of these components can reveal the desired information about the animal geographical origin and/or the rearing system. Using this approach, Baroni et al. [82] were able to differentiate meat samples coming from three different cattle-producing regions in Argentina. In the work of Coletta et al. [83], isotope ratios of carbon and nitrogen were used to reveal the feeding systems used to rise chickens (barn vs. free-range). However, it is also true that the effectiveness of these methods is not satisfactory in all cases, and success will largely rely on the sensitivity and accuracy of the determinations, especially at the time to differentiate meat samples coming from neighboring geographical areas[79].

The increase of water content with the objective to artificially step up weight and price is between the most common practices of meat adulteration. The classical method to detect this fraud was to determine the water/protein ratio. However, addition of water can be masked by addition of exogenous proteins and phosphate, since this addition would leave the water/protein ratio close to the original values [81]. Thus, alternative methods such as nuclear magnetic resonance have been developed for this purpose, since they have proved to be a good way to study the water distribution in meat [84]. These techniques have proved to be also effective in both detecting the addition of substances to increase the water holding capacity of meat [85] and in differentiating fresh from thawed meats [86].

Infrared spectroscopy has been applied to meat authentication as a fast and non-destructive technique capable to differentiate meats from different farm animals such as beef, lamb, pork and chicken [87] and also from less common meat species such as horses and llamas [88]. Recently, Fourier transform infrared spectroscopy (FTIR) in conjunction with multivariate analysis and soft independent modelling class of analogies (SIMCA) has been successfully applied to identify the adulteration of minced beef meat with horse meat, fat beef trimmings and soy protein. According to authors, with this technique it was also possible to quantify the percentage of adulteration, showing a good correlation with the absorbance values obtained in the Mid-Fourier transform spectrum [89]. Additional advantages of the infrared spectroscopy approach would be the need of small sample volumes and the fact that minimal or no sample preparation is required. On the other hand, it is not always easy to get unambiguous results with those determinations since the measured parameters are frequently common to the different meats or adulterants and so there is need for quite robust discriminative data analysis.

Metabolomics is another interesting and powerful approach used in the detection of meat frauds and meat authentication issues. The objective of this strategy is the identification and quantification of as many low molecular weight compounds as possible that can contribute to differentiate samples. For the analysis of metabolites, the use of chromatographic techniques such as gas chromatography coupled to different detectors such as flame ionization detector (FID) or mass spectrometry (MS) has been described. An example is the work carried out by Surowiec et al. [90] where authors were able to detect the presence of mechanically recovered meat (MRM) from different pork meat samples by using GC-MS metabolite profiling. Principal component analysis of analyzed metabolites allowed differentiating mechanically recovered meat samples from both hand-deboned and desinewed meat. However, such a good discrimination is not always possible with this kind of approaches. An alternative to GC-MS in determining volatile compounds for authentication purposes has been reported by Nurjuliana et al. [91] with the use of electronic nose as a rapid, affordable and non-destructing method for the detection of pork meat in *halal* meat samples. With difference to GC-MS, this instrument would integrate measurements of the total headspace volatile compounds, generating an aroma pattern capable to establish differences or similarities between samples but without identifying individual compounds. Authors stated that this detector would have some advantages such as high sensitivity, easy to use and long term stability.

As a general rule, all the approaches commented previously are based in the generation of large amount of data and chemometric analysis, as a way to establish differences within the chemical composition of samples. However, since muscle and meat exhibits a large natural variation due to multiple factors (species, breed, sex or slaughter age, for instance), analysis and comparison of data can become particularly complex, affecting the reliability of results. As discrimination is based on a general analysis of elements and metabolites, authentication is frequently a matter of trends or differences that are far from being clearly discriminant and conclusive.

An alternative to these non-targeted approaches based on overall measurements consists on searching for clearly defined biomarkers capable to provide the necessary information about the presence of a particular meat component and/or adulterant. In these targeted approaches, the detection of marker proteins has been traditionally used to specifically detect the different meat species and tissues, together with protein additions or substitutions. Among a variety of techniques [92,93], the use of immunoassays has been notably widespread for authentication purposes. Immunoassays have undoubtedly some interesting advantages, such as easy to use by non-specialized staff, high sensitivity and the capacity to process a high number of samples in short times [94]. But they are not exempted from some important limitations such as the need for specific antibodies. If antibodies are not highly specific, problems associated to cross-reactions can occur, especially in differentiating between closely related species, as in the case of chicken

and turkey [95]. Immunoassays can also have limitations in the analysis of processed meat foods because processing can alter protein structure and thus negatively affect the recognition of the target protein by the antibody. In that respect, efforts to try to overcome these limitations have been carried out with the development of antibodies raised against thermostable proteins [96].

More recently, the development of methods based on DNA analysis have been promoted with the objective to overcome the commented limitations on protein identification. As main advantages, DNA-based analyses are highly sensitive and have a considerably higher discrimination capacity because they are based on identifications of targeted DNA sequence fragments that are unique of a particular species or tissue. This is the reason why they have become so popular for the unambiguous identification of meat species. This would be also applicable to the identification of closely related species or even in identifying different breeds of a particular species. Such high discriminating power would be especially interesting in the authentication of traditional and regional meat products, since during the manufacturing of these products specific animal breeds characteristic of one particular geographic area are employed. In the work carried out by Fajardo et al. [97], they were able to qualitatively differentiate between domestic pork and wild boar meat. This is not always the case, and so the routine identification of very close related species, breeds or strains still remains a matter that is far to be solved [79]. The use of methods based on DNA analysis can display some important limitations in meat foods, as in the case of processed meat products. As foods are complex matrices, it is difficult to develop standardized extraction protocols, being necessary to optimize them for each particular case to ensure that reproducible amounts for the analysis of the different samples are obtained and that inhibitors of DNA amplification are eliminated [98]. During the processing of meat products, DNA can undergo an important degradation because of disruption of cellular integrity and the liberation of hydrolytic enzymes, heat treatments or pH changes, for example. This can reduce the length of DNA fragments that can be amplified to implement PCR analysis and consequently increasing the possibilities of cross-reactivity with other species. In such cases, specific identification should be based on the study of short DNA sequences of maximum few hundred base pairs [79]. All these limitations become especially important when quantitative determinations are required. Quantitative DNA analyses should be based on the analysis of single-copy DNA. However, this does not allow such a so low limit of detection as the one obtained from amplification of genes having multiples copies. Using only single copy genes reduces the probability to find highly specific sequences, which can affect the specificity of the assay [99].

From what it has been reviewed, it can be inferred that there is need to develop alternative approaches capable to overcome the existing limitations of the methods currently in use for meat authentication. The revolution carried out since the 1990s up to date on the analysis of peptides and proteins with the advent of the so called “soft ionization” techniques (MALDI and ESI) coupled to mass spectrometry, has open a new way to understand the use of these biological compounds as reliable biomarkers for the identification of species and tissues. More concretely, analysis of peptide sequences that are present in foodstuffs either as such or generated by enzyme digestion of food proteins would display a high discriminating power comparable to methods based on DNA analysis. Interestingly, peptides would show higher resistance than DNA sequences to the degradation caused by food processing techniques. Consequently, the development of methods based on the analysis of peptides and proteins by mass spectrometry has great potential in meat authentication studies, especially in the analysis of highly processed meat products. Also, the extraction of proteins and peptides from food matrices would be more feasible as compared to DNA extraction, so that standardized extraction protocols can be proposed. The level of performance of current mass spectrometers is of great help at the time to accurately determine the sequence of target peptides even if present at very low amounts. All together, these facts would allow considering the peptidomic approach for the development of accurate, sensitive and reliable quantitative analytical methods.

Mass spectrometry has been successfully applied in the identification of different antibiotic-resistant species of bacteria susceptible to contaminate the different steps of the food production system. In the work of Huber et al. [100], the identification of methicilin-resistant coagulase-negative staphylococci using MALDI-TOF MS was concluded to be a fast and reliable tool for the screening of large sample amounts to assess the prevalence of this infection in livestock production. Detecting the presence of allergenic proteins is also a matter of major importance in food safety. Thus, development of different peptidomic strategies has been reported in the literature as an effective way to detect these proteins in different food matrices such as peanuts [101] or lupin [102], for example. Recently, Carrera et al. [103] have developed an interesting workflow for the rapid and efficient detection of the major fish allergens, parvalbumins, through the rapid purification of these proteins followed by an accelerated in-solution protein digestion and screening of a few number of peptide biomarkers by selected MS/MS ion monitoring. According to authors, this is the fastest method reported for the direct detection of these allergens.

The potential of mass spectrometry to undertake effective and reliable species identifications was already suggested about twenty years ago by using the mass difference of haemoglobins and myoglobins as discriminative criterion [104]. Using this strategy on a single quadrupole mass spectrometer, Espinoza et al. [105] took the obtained molecular weight values for α- and β-haemoglobin chains as biological markers to try to discriminate 62 animal species, mainly birds and mammals. In this way, they were able to specifically identify 86% of the total evaluated samples. The rest of values, however, were not able to identify the corresponding species specifically, showing overlapping with other species. In another work two related species of sperm whales (*Kogia breviceps* and *K. sima*) were efficiently differentiated using this same approach [106].

The discriminating capacity of the proteomic approach in species authentication can be increased when, instead of using molecular mass determinations, peptide sequence tags specific of each animal species are used as targeted biological markers. In this kind of strategies, selected proteins are normally subjected to enzymatic digestion with trypsin or any other

type of endopeptidase, being the generated peptides sequenced by tandem mass spectrometry (MS/MS). Comparative sequence alignment allows selecting those peptides that are specific of a particular animal or vegetal species. As the identification criterion is made at sequence level, the peptidomic approach in terms of discriminative power will be comparable to those based on DNA analysis. This can be observed in the work of Pascoal et al. [107], where a combination of genomic and peptidomic approaches was developed to identify a particular shrimp species (*Pandalus borealis*) in a variety of foodstuffs containing different prawn and shrimp species either as whole individuals or in processed products. Both approaches succeeded in the identification of this species. However, the identification of species-specific peptides through the peptidomic approach would open the possibility to the design of fast and easy-to-use detection methods.

In the last years, there has been some changes in the legislation concerning the use of animal proteins to feed other farm animals. This practice was prohibited by the European Commission with the aim to halt the spread of the bovine spongiform encephalopathy (BSE). As the number of BSE cases has progressively decreased during the last years, some lifting of this prohibition has been considered for certain classes of animal proteins. However, this opening requires strict surveillance in order to assure that abuse and illegal practices do not arise from feed producers. To do that, robust, accurate and sensitive methodologies are needed. Balizs et al. [108] have recently reported the development of a proteomic strategy to detect the presence of meat and bone meal (MBM) in foodstuffs together with its animal origin, using osteocalcin as the target protein. The developed procedure comprised extraction of proteins from pulverized bone, followed by concentration of the extract. In a first approach, the concentrated protein extract was cleaned up with C_{18} pipette tips as a step prior to direct analysis by MALDI-TOF MS. This allowed determining the molecular mass of intact bovine and porcine osteocalcin, being the mass differences between them sufficient to allow discrimination between these two species. However, this was not so evident in the case of MBM subjected to heat treatments higher that 133 °C because of the important degradation suffered by osteocalcin at such temperatures, especially in the case of bovine species. To solve this problem, they proposed an alternative peptidomic strategy consisting on different purification steps followed by in-solution trypsin digestion of osteocalcin and identification of marker peptides by either MALDI-TOF or LC-ESI-Q/TOF MS. The use of this latter high resolution mass spectrometer allowed an increased sensitivity at the time to detect osteocalcin in processed samples, together with unambiguous identification of both bovine and porcine marker peptides by MS/MS peptide sequencing. Despite these refinements, some problems persisted for detecting osteocalcin marker peptides in MBM heated at 141 °C in the case of bovine species.

A peptidomic approach has been recently described for the specific detection of chicken meat in mixes with other types of meats [109]. The method is reported to be robust, reliable and sensitive, allowing the detection of as low as 0.5% chicken in meat mixes. The peptide sequences DQGTFEDFVEGLR (Mr. 1512.6 Da) and ALGQNPTNAEINK (Mr. 1369.5 Da) were generated after trypsin hydrolysis of chicken myosin light chain 3 and used as discriminative criterion to unambiguously report the presence of chicken meat in both raw and cooked meat samples. Identification of these chicken-specific sequence tags was initially carried out by MALDI-TOF MS and peptide mass fingerprinting. The amino acid sequences corresponding to these peptide masses were further confirmed by MS/MS using a conventional LC-ESI-ion trap instrument (See Fig. 2).

In this case, the aggressive heat treatment applied to produce the cooked meat samples (180 °C during 1 h) did not affect the quantitative detection of these peptides, thus allowing a good linearity between the amounts of the detected biomarker peptide and the amount of chicken meat present in the sample. The use of affordable mass spectrometry equipment would also facilitate the implementation of this peptidomic strategy to laboratories in charge for controlling meat frauds, representing a serious and complementary alternative to methods currently in use for meat authentication and detection of frauds, such as immunoassays or PCR-based analyses. The capacity of peptidomic-based analyses at the time to discriminate between closely related meat species would be comparable to methods based on DNA analyses because differentiation is also done at sequence level. A proof of this can be seen in the application of this technology to differentiating chicken and turkey meats [110]. The high discriminative power of the peptidomic approach is also well illustrated in the work of Buckley et al. [111]. These authors reported a method to isolate and characterize, using MALDI-TOF/TOF MS, a 33 amino acid collagen peptide containing sequence differences between sheep and goat species, allowing determining the species type in archaeological bones. The work highlighted the high stability of peptide material in comparison to other biomolecules such as DNA, in accordance to the idea that peptidomic analyses would have great potential in determining meat compositions on highly processed foods.

5.2. *Proteomics, peptidomics and meat frauds—concluding remarks*

The ability to differentiate not only between closely related species but also between individual breeds of the same species represents an additional and even more complicated challenge in the authentication of meat products. There are several reports that have tried to accomplish this task by making use of the proteomic technology, as mentioned in Section 2.1. Nevertheless, even if this seems to be a promising strategy to differentiate between animal breeds, we must keep in mind that this strategy would be adequate in authentication studies of pure samples. Additions/replacements of meats with different animal breeds, as it can be the case of frauds in traditional and regional meat products, would be much more difficult to resolve. Despite the great advances in proteomic and peptidomic technologies during the last years, its application to meat authentication and detection of frauds in meat and meat products has been quite limited up to date. As we can see, potential exists for mass spectrometry applied to protein and peptide analysis to become an interesting and complementary alternative to methods currently in use for meat authenticity, but still much work is needed to solve the commented limitations and to provide suitable protocols, adequate for control laboratories.

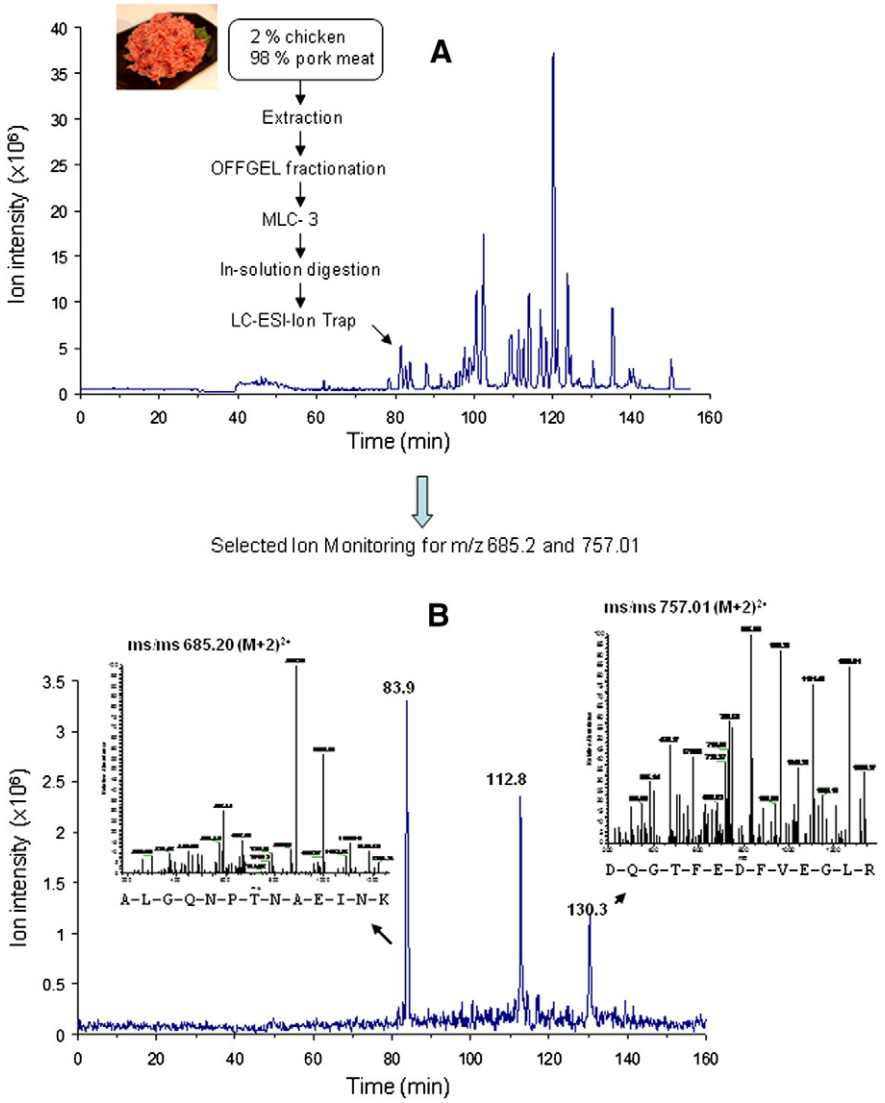


Fig. 2 – A brief summary of the Peptidomic approach developed by Sentandreu et al. [109] to detect the presence of chicken meat in a mix with other types of meats. In this particular case, proteins from a mix of meats containing 2% chicken in 98% pork meat were extracted according to the protocol. The peptide mixture obtained after in-solution trypsin digestion of fractionated myosin light chain 3 (MLC-3) was separated on a LC-ESI-Ion trap instrument (Thermo ® LCQ) using a Clipeus C18 column (150×0.5 mm i.d.) as shown in part A. Part B shows the selected ion monitoring for m/z values 685.2 (83.9 min) and 757.01 (130.3 min), corresponding to the chicken-specific peptide sequences ALGQNPTNAEINK and DQGTFEDFVEGLR, respectively. The presence of these peptide signals in the chromatogram indicates thus the presence of chicken meat in the original sample. The peptide signal appearing at 112.8 min corresponds to a sequence common to the porcine species and consequently not suitable for use as a chicken-specific peptide biomarker.

6. Proteomics, meat and microbial contamination

Meat is an excellent substrate for bacterial growth and if some methods to restrict their presence are not applied, meat becomes contaminated. The characteristic microbial populations that develop in meat and meat products are the result of the effect of the prevailing environmental conditions. The intrinsic and extrinsic factors governing microbial growth will determine the type and number of bacteria present in meat, these factors being predominantly chemical (concentration and availability of nutrients, pH, redox potential, buffering capacity, aw, meat structure) and related to storage and processing conditions. The extrinsic factors are often manipulated to extend the shelf life of meat products, temperature and oxygen availability being the major parameters. Hygiene during slaughter and dressing of carcasses together with prompt and adequate cooling are of major importance for meat quality and safety.

Carcass decontamination strategies using sanitizing solutions have been successful applied for pathogen reduction in beef carcass, especially when combined with pre-chill treatments [112]. In response to consumer's demands related with food free from pathogens, with minimal processing and fewer

preservatives and additives, but keeping its sensorial quality, current trends in the food industry include the investigation of alternative inhibitors. Especially, biopreservation has gained increasing attention as a means of naturally controlling the shelf life and safety of foods. Certainly the use of bioprotective cultures to ensure the hygienic quality of food is a promising tool being Lactic Acid Bacteria (LAB) the preferred bacteria for bioprotective and starter culture formulation. Indeed research of this technological potential has been of industrial outmost interest.

Salmonellae are typically intestinal pathogens and represent an important organism of public health significance. High levels of Salmonella in the meat may arise for animal production practices at the rearing stage as well as cross-contamination after slaughter [113]. Food-poisoning staphylococci are also widely distributed; meat contamination being generally associated with highly manual handled foods and for the most part, the etiologic agent is *Staphylococcus aureus* and its related heat stable enterotoxins [114]. Enterohemorrhagic *Escherichia coli* shiga toxin-producing (STEC), emerged as a foodborne pathogen more significant than other well-known ones because of the severe consequences of infection, its low infection dose, its unusual acid tolerance and its apparent special but inexplicable association with ruminants used for food [115]. On the other hand, *Listeria monocytogenes* has continued to raise food safety concerns, especially with respect to ready-to-eat (RTE) products. Listeriosis, caused by this pathogen, is a significant public-health concern as a result of its clinical severity and high mortality rates [116]. Species within the genus *Campylobacter* and *Yersinia* have also emerged as pathogens of human public health concern [117]. Other pathogens of human health concern that may be present but remain undetected in slaughtered animals involve streptococci, clostridia and *Corynebacteria*. Cold storage of meat will decrease bacterial growth; only 10% of the bacteria initially present being able to grow at refrigeration temperatures. Oxygen restriction by the use of vacuum or modified atmospheres will drastically reduce the presence of Pseudomonas and bacterial flora will be gradually selected towards CO2-tolerant organisms. Under these conditions the dominating microorganisms involves *Brochothrix thermosphacta*, *Enterobacteriaceae* and LAB.

Fermented dry sausages are known as "shelf stable products", this term referring to those products that do not require refrigeration or freezing for safety and acceptable organoleptic characteristics. LAB and Gram-positive, coagulase-negative cocci (GCC) are the microorganisms primarily responsible for sausage fermentation. It is well known that LAB, in particular lactobacilli, plays an important role in meat fermentation processes. They contribute to the hygienic and sensory quality of meat products mainly through carbohydrates and proteins catabolism resulting in pH reduction, production of antimicrobial agents such as organic acids, inhibitory peptides or bacteriocins and generation of flavor compounds [118]. On the other hand, GCC participate in color development and stabilization through a nitrate reductase activity and their antioxidant potential due to catalase activity [119]. Even though, raw dry sausage materials (meat and casings) are the principal vehicles for pathogens and contaminating microorganisms. *S. aureus*, Salmonellae and *Clostridium perfringens* have been traditionally implicated in fermented dry sausage contamination [120]. The emergent pathogens within the genera *Campylobacter* and *Yersinia* as well as *L. monocytogenes* and STEC *E. coli* have also been involved in outbreaks caused by fermented sausage [121].

6.1. Proteomics as a tool to understand the molecular basis of bacterial physiology

Traditionally, biochemical studies of microorganisms of technological interest have been focused on metabolic pathways or biosynthetic capacities of economically important species. Medical and food safety research areas are interested in the identification of immunogenic proteins that may be potential vaccine targets as well as in extending the understanding of antibiotic action. All these research purposes require a holistic approach rather than restricted investigations focused on single or small groups of genes or proteins, as before genomics development. The advanced information provided by the genome sequencing projects as well as the progresses in protein identification analysis has permitted to get insights in the molecular basis of bacterial behavior. As well as, in the study of beneficial and pathogenic bacteria the combined technologies of genomics, proteomics and bioinformatics has provided valuable tools for the study of complex phenomena determined by the action of multiple gene sets. In the past, proteomic approaches were rather descriptive being focused on protein identification. More complete studies combining proteomics, transcriptomics and metabolomics are bringing new clues for understanding bacterial physiology. Until recently, the study of global protein expression was performed almost exclusively using 2-DE, a technique developed in the 1970s [122] with significant advances in the last decades. However, it is well-established that 2-DE methodology has several limits such as difficulty to detect poorly expressed proteins; gels can only focus proteins in the pI range of 3–10 [123]; limitation of protein size resolution since only 10–200 kDa proteins can be detected; and restriction in the analysis of certain proteins due to their low solubility (e.g. membrane proteins) [124]. Nevertheless, gel-based proteomics is still a powerful tool to address many physiological topics because (i) most of the metabolic pathways can be visualized on 2-DE gels, (ii) stress responses can be directly followed because gel-based proteomics can discriminate between protein synthesis (visualized by ^{35}S-methionine pulse labelling) and protein level accumulated in the cells, and (iii) post translational modifications or protein damage, and even proteolysis at a proteome-wide scale can be visualized. MS-based procedures, on the other hand, are absolutely required to cover the entire proteome including membrane proteins or low-abundance proteins. In this section, we will illustrate that a combination of gel- and MS-based approaches is essential to visualize the entire proteome of bacteria in order to address physiological questions such as adaption and tolerance to stressful environments, construction of proteome data bases or pathogenesis directed studies.

6.2. State-of-the-art of proteomics in meat- and meat product-related bacteria

When bacteria are subjected to a sudden shift in one or several parameters affecting their growth or survival, a program of gene expression is initiated, which is displayed

as an increased or decreased amount of proteins synthesized in response to stress. Different stress conditions such as high salt concentration, wide range of pH, low water availability and low temperatures provoke pleiotropic effects on cell physiology. The knowledge of the adaptive mechanisms will facilitate the selection of the most efficient strains to be used as starter or bioprotective culture for a particular product. In the case of pathogens, the high degree of adaptability of some bacteria such as Listeria is one reason for the difficulty in controlling them in meat products since treatments used in food processing and preservation often utilize stressing agents and parameters to which they are resistant. For this reason the insight in the adaptive mechanisms of bacteria will contribute to the development of new approaches for controlling meat contamination. In addition, as mentioned before the use of sanitizers for controlling food contamination is an effective strategy usually employed in the meat industry, the comprehension of the nature of bacterial response to biocide treatment, and the associated mechanisms involved, could ultimately facilitate the refining and optimizing of biocide formulations to overcome potential tolerance mechanisms, leading to an improvement in biosecurity measures and further enhancing the protection of farm-animal and thus, public health. The most recent proteomic studies reported in bacteria related to meat environment are discussed in the following paragraphs and summarized in Table 1.

6.2.1. *Pathogens*

6.2.1.1. L. monocytogenes. Pioneering studies using two dimensional electrophoresis and different biological techniques investigated adaption of this bacterium to different stress effectors such as high salt concentration, high/low temperatures and acid/alcali environments [136–138]. Furthermore, proteomics has been applied to better understand biofilm formation [139] and to study pathogenic factors in this microorganism [140–142].

Cold stress resistance and low-temperature growth capability of some psychrotolerant bacteria are biological properties mediated through many molecular response mechanisms at the microbes' disposal to maintain membrane structural integrity, to ensure nutrient uptake, to retain ribosome functionality and to face inefficient or slow protein folding, reduced enzyme activities, decreased ability in DNA replication and transcription. Cacace et al. [143] investigated, by means of expression proteomics, cold adaptation mechanisms which allow *L. monocytogenes* to grow at 4 °C, a condition resembling that of refrigerated foods. Results showed increased level of chaperones, folding catalysts, transporters for osmolytes and oligopeptides uptake, proteins known to be involved in bacterial response to several stress conditions (such as catalase and superoxide dismutase) and in energy production, necessary to sustain cold growth. Moreover, the expression of the transcription regulator Catabolite Control Protein A (CcpA) is also increased in cold adapted cells. CcpA is known to be a central regulatory element of carbon metabolism and of other metabolic pathways in gram-positive bacteria [144]. Authors suggested a chaperone-like function in protein folding and protection against thermal denaturation to elongation factors, as reported also in *E. coli* [145]. Although, to assist in designing *L. monocytogenes* mitigation strategies for ready-to-eat food products, further studies are necessary to specifically evaluate the effects of food composition, additives, preservatives, and processing technologies on the modulation of *L. monocytogenes* cellular components in response to specific stresses. Other interesting proteomic approach carried out in *L. monocytogenes* is the study of signal transduction. Nowadays, a bulk of evidence raised from genome sequence data indicates that Ser, Thr, and Tyr phosphorylation (characteristic of eukaryote signaling) is also widespread in prokaryotes [146]. These eukaryotic-like signaling systems have been shown to control essential processes in bacteria, including development, cell growth, stress responses, central and secondary metabolism, biofilm formation, antibiotic resistance, and virulence [147]. In particular, it was reported that the *stp* gene (lmo1821) encodes a functional Ser/Thr protein phosphatase (STPP) required for *L. monocytogenes* growth and virulence in murine model of infection. Lima et al. [148] carried out an interactomic approach, where 62 proteins that possibly interact directly or indirectly with the phosphorylated catalytic domain of PrkA, a putative transmembrane Ser/Thr protein kinase (STPK) were identified. The diversity of the identified interacting proteins suggests that the signal transduction pathways mediated by this STPK in *L. monocytogenes* may affect a large variety of fundamental biological functions including protein synthesis, cell wall and carbohydrates metabolism.

6.2.1.2. E. coli STEC. Among the most recent reports applying functional genomics for the study of *E. coli* STEC and meat processing conditions, it can be mentioned the one by Kocharunchitt et al. [149] who studied the physiological response of *E. coli* O157:H7 Sakai to low temperature and water activity conditions skilled during meat carcass chilling. The response of *E. coli* during exponential growth at three different conditions (14 °C, a_w 0.985; 25 °C, a_w 0.967; and 14 °C, a_w 0.967) was compared with that of a reference culture (35 °C a_w 0.993). Gene and protein expression profiles of *E. coli* were more strongly affected by low water activity (a_w 0.967) than by low temperature (14 °C). Results showed that a universal response of *E. coli* to all test conditions included activation of the master stress response regulator RpoS and the Rcs phosphorelay system (involved in the biosynthesis of the exopolysaccharide colanic acid), as well as down-regulation of elements involved in chemotaxis and motility was registered. Another study involving phenotypic and proteomic analyses using an *E. coli* O157 (EHEC) strain (B-1) and a commensal *E. coli* K-12 strain, exposed to prolonged cold stress, revealed that the pathogenic strain was significantly more resistant to cold stress than the non-pathogenic *E. coli*. The results of this study also demonstrated that the RpoS sigma factor plays a significant role in growth response of the *E. coli* O157 strain to cold stress. Through examination of the rpoS mutant proteome profile, a more pronounced action of RpoS in regulating enzymes involved in the modification of membrane lipid bilayers of cold adapted cells was proposed. In addition, RpoS seems to be responsible in promoting resources (energy, protein synthesis, etc.) during proliferation of the cold adapted cells. This work highlighted that *E. coli* O157 strains possess unique mechanisms enabling survival and proliferation under low temperature conditions relative to non-pathogenic *E. coli* strains [150]. According to this, King et al. [151] determined that *E. coli* O157:H7 likely has a greater capacity to survive in more complex acidic environments when

Table 1 – Recent proteomic reports on bacteria related to meat and meat products.

Bacteria	Objective	Results	Reference
Pathogen			
Listeria	Analysis of *L. monocytogenes* protein expression by grown on RTE sliced turkey meat	Up regulated proteins on meat matrix were related to virulence/stress adaptation and surface antigen.	Mujahid et al. [125]
E. coli	Comparison of protein profiles, following growth in cell culture or bacterial isolation from intestines of infected piglets assessed by a shotgun proteomic approach.	Quantification of more than 2500 proteins. The ORFs encoded by phage/prophage regions were expressed at the protein level only at 13%. Coverage of proteins producing functional virulence factors was markedly higher, 31 and 36% for proteins encoded by plasmid pO157 and the LEE pathogenicity island, respectively.	Pieper et al. [126]
Staphylococcus	Revision of the impact of 2D gel- and mass spectrometry-based proteomic approaches on pathophysiology and virulence of *S. aureus*	Discussion about proteomic signatures at different stress conditions and virulence factors in *S. aureus* secretome. The combination of both techniques allows a proteomic view of all subproteomic fractions as well as posttranslational processes.	Hecker et al. [127]
Salmonella	Study of the tolerance to triclosan, a phenolic broad spectrum antimicrobial compound. A triclosan sensitive *Salmonella enterica* serovar Typhimurium and the isogenic triclosan tolerant mutant are compared	The triclosan tolerant Salmonella mutant, ST23M, expressed increased amounts of FabI. Triclosan exposure induced multiple cellular modifications in the proteome of both the parent and mutant isogenic strains (up-regulation of proteins related to general and oxidative stress, cellular metabolism, permeability and transport and quorum sensing). The mutant employs similar defence network as the parent isolate, although with a divergent triclosan defence response involving up-regulation of the FabI target and efflux activity	Condell et al. [128]
	Understanding the control of virulence genes by guanosine tetraphosphate and guanosine pentaphosphate (pppGpp) under starvation, in *S. Typhimurium* wild-type and *ppGpp*^{0} mutant strains	2-DE reference map of amino-acid starved *S. Typhimurium* is achieved. As well as the identification of a novel virulence-associated factor, STM3169 regulated by ppGpp and by an SPI-2 two-component regulator, SsrB.	Haneda et al. [129]
Campylobacter	Study of cell response to Ultra high pressure (HP) injury and subsequent recovery	Main pathways involved in HP response were identified: Citrate cycle; amino acid metabolism; gluconeogenesis; fatty acid biosynthesis and proteins related to oxidative stress	Bièche et al. [130]
	Proteomes of *C. jejuni* grown under standard growth conditions or that obtained from within epithelial cells were compared.	Inside the cells, *C. jejuni* undergoes a significant metabolic downshift and reprograms its respiration, favoring the respiration of fumarate.	Liu et al. [131]
Bacteria with technological interest			
Lactobacillus	Analysis of *Lb. sakei* 23 K proteome differentiation in the presence of meat extracts	Overexpression of peptidases and proteins related to energy and pyrimidine metabolism, translation, while others corresponding to general stress response, pyrimidine, vitamin and cofactor biosynthesis were down-regulated in the presence of meat proteins	Fadda et al. [132]
	Understanging adaption of *Lb. sakei* CRL1756, an anchovy isolate, to salted environments in the presence of osmoprotectants	Improved tolerance to 10% salt was achieved in the presence of Glycine-Betaine. The induction of a DyP-type peroxidase (involved in inorganic ion transport and detoxification mechanisms) and decreased synthesis of glycolytic enzymes under hypertonic stress. Malate dehydrogenase and pyruvate oxidase were induced as an alternative energetic pathway for cellular maintenance in salting environment.	Belfiore et al. [133]
S. xylosus	Fractionation and analysis of cell envelope proteins of *S. xylosus* C2A. Comparison between planktonic and sessile proteomes	Ninety distinct cell envelope proteins were identified, some of them with multiple subcellular localization. Amino acid biosynthesis, protein translation, and protein secretion were up-expressed in biofilm, suggesting a more active protein trafficking in sessile cells. Proteins involved in exopolysaccharide (EPS) biosynthesis were up regulated in biofilm.	Planchon et al. [134,135]

comparing an *E. coli* O157:H7 and a non-pathogenic *E. coli* K-12 strain. These works help to elucidate molecular basis of cold adaption of this pathogen, thereby enabling the formulation of new measures specific for low-temperature food-processing and storage conditions, as well as its elimination or control[150].

6.2.1.3. Salmonella. Concern has been expressed about the overuse of biocides in farm animal production and food industries. Biocide application can create selective pressures that lead to increased tolerance to one or more of these compounds and are concomitant with the emergence of

cross-resistance to antibiotics. A *Salmonella enterica* serovar Typhimurium (S. Typhimurium) strain sensitive to triclosan (phenolic compound with broad antibacterial spectrum) and the isogenic triclosan tolerant mutant were studied at the proteomic level in order to elucidate cellular mechanisms that facilitate biocide tolerance. DIGE compared protein profiles of parent and mutant Salmonella strains, in the presence and absence of triclosan. Differentially expressed proteins were identified and divided into two groups: Group A describes proteins differentially expressed between susceptible and triclosan tolerant Salmonella and includes the known triclosan target FabI which contained a mutation at the triclosan target binding site. Group B identified proteins differentially expressed in response to triclosan exposure and defines a general cell defence network. Only four proteins were common to both groups highlighting the diverse range of pathways employed by Salmonella to counteract biocides. These data suggest that sub-lethal concentrations of triclosan induce discernible changes in the proteome of exposed Salmonella and provide insights into mechanisms of response and tolerance [128]. Other interesting work focused on the role of guanosine tetraphosphate and pentaphosphate (ppGpp) on the pathogenesis of bacterial infections is described herein. It is known that in *S. enterica* serovar Typhimurium several genes, including virulence genes, are regulated by ppGpp when bacteria are under starvation. To understand the control of virulence genes by ppGpp a comprehensive 2-DE reference map of amino acid-starved *S.* Typhimurium strain SH100 was established. The comparative analysis of the wild-type and ppGpp0 mutant strains revealed 55 proteins which expression was affected by ppGpp. Using a mouse infection model, a novel virulence-associated factor, STM3169, from the ppGpp-regulated and Salmonella-specific proteins was identified. Furthermore, the expression of stm3169 was controlled by ppGpp and SsrB, a response regulator of the two-component system located on *Salmonella* pathogenicity island 2. This work demonstrated that proteomics by using a 2-DE reference map is a powerful tool for analyzing virulence factors and the regulatory network involved in *Salmonella* pathogenesis [129].

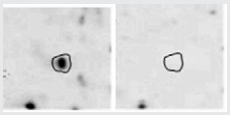
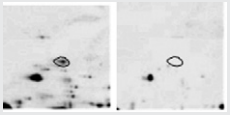
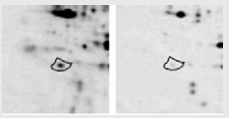
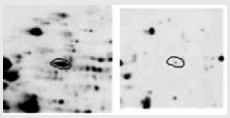
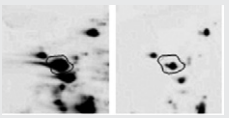
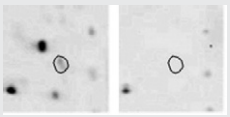
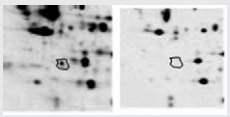
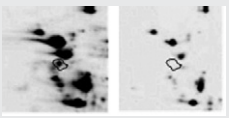
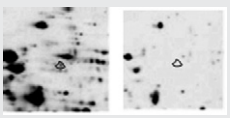
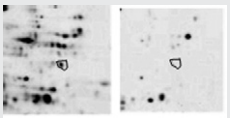
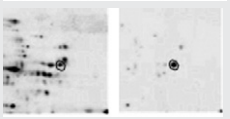
6.2.2. *Bacteria of technological interest*

As mentioned before, LAB are the preferred bacteria for starter culture formulation for meat and other raw materials (milk, vegetables, and cereals). Ideally, appropriate cultures have to be selected from indigenous microorganisms, in order to be more competitive, well-adapted to a particular product, and with high metabolic capacities to beneficially affect quality and safety of the product and preserve their typicity [152]. As mentioned before, the study of bacterial adaption to the different processing conditions is focus of study also on this type of microorganisms. Proteomics represents a challenge for scientific analysis and opens new perspectives for bacterial starter research purposes [153]. New findings about the molecular basis of adaption and technological potential of bacteria related to meat fermentation are described as examples of the convenience of the omic approaches.

6.2.2.1. Lactobacillus sakei. *Lb. sakei* is recognized as one of the most important components of starter cultures used for production of fermented meat products[132]. The complete genome sequence of the strain *Lb. sakei* 23 K, a psychrotrophic LAB naturally present in fresh meat, showed the existence of a specialized gene catalog related to its ability to survive and compete in meat environments [154]. *Lb. sakei* 23 K is the best characterized meat born strain from a proteomic approach. By applying the omic technologies some of the above mentioned functions have been proven such as proliferation at refrigeration temperatures and high salt concentrations (up to 9% sodium chloride) [155], thereby confirming the predicting value of genome data mining. In this sense bacterial cells have developed strategies for their acclimation to osmotic stress such as the intracellular accumulation of compatible solutes (glycine-betaine, proline, trehalose, and glycerol), which equilibrate cellular osmotic pressure [156]. Subsequently, a long-term process occurs along which a program of gene expression is initiated [136]. Recently Belfiore et al. [133] studied the adaption of the anchovy isolate *Lb. sakei* CRL1756 to salted environments in the presence of osmoprotectants. Glycine-betaine (GB) pre-treated cells exposed to 10% NaCl showed improved adaption, achieving growth after a long lag phase while cell growth was not observed in the salted medium without GB. Proteomic analyses revealed decreased synthesis of some glycolytic enzymes and induction of some other: the malate dehydrogenase (MleS) and pyruvate oxidase (Pox2), related to an alternative energetic pathway. Proteins belonging to general stress response and nucleotide metabolism were up-regulated. Noticeably, the induction of DyP-type peroxidase, involved in iron transport, detoxification and oxidative stress, was observed in this strain under osmotic constraint (Table 2).

The addition of GB as compatible solute was crucial for *Lb. sakei* CRL1756 to overcome this major osmotic constraint. To counteract the decreased synthesis of glycolytic enzymes under hypertonic stress, *Lb. sakei* CRL1756 was able to induce the enzymes MlesS and Pox2 as an alternative energetic pathway for cellular metabolism maintenance. The natural presence of GB in fish guarantees better adaption of *Lb. sakei* CRL1756 to salted environment ensuring its robustness and stability as starter culture for salted anchovy products [133]. On the other hand, Fadda et al. [132] carried out a physiologic and proteomic study to unravel metabolic strategies of *Lb. sakei* 23 K for the adaptation to meat environment. In this study, meat myofibrillar proteins were observed to exclusively regulate the expression of proteins related to energy, lipid and amino sugar metabolisms, cofactor and vitamin biosynthesis and cell wall formation, while cellular processing and signaling proteins were modulated only by sarcoplasmic extracts. Notably the proteins related to stress were found to be synthetized in lower amounts. Thus meat proteins would not represent a stress environment per se for *Lb. sakei* 23 K, in contrast to the harsh conditions during meat processing (cold, salt, redox potential variations) [132]. In other proteomic study 10 isolates of *Lb. sakei* from different meat and fish origin were compared. The study focused on the glucose and ribose metabolisms, the main sugars available in meat and fish. Results showed that protein expression varied according to the type of sugar present in the medium. Interestingly, a commercial starter culture and a protective culture strain down-regulated the glycolytic pathway more efficiently than the rest of the strains when grown on ribose. Also, the strain isolated from fermented fish showed a higher expression of stress proteins growing on both carbon sources [157].

Table 2 – Examples of main identified proteins of *L. sakei* CRL1756 significantly modified during its growth in MRS + GB + 10% NaCl.

Protein	Function	Fold (Salt/Control)	Salt	Control
Hsp20	Stress response	8.8		
ClpB	Stress response	4.4		
Chaperone GrpE	Stress response	2.0		
ClpL ATPase protein	Stress response	2.9		
Chaperone DnaK	Stress response	1.5		
Ferritin-type DNA-binding protein	Atypical conditions adaptation	2.4		
Dyp-type peroxidase	Inorganic ions transport and detoxification	1.8		
Malate dehydrogenase	Glycolysis	2.6		
Pyruvate oxidase	Carbohydrates metabolism	2.3		
Adenylosuccinate synthetase	Nucleotides/nucleic acidsmetabolism	2.5		
Arginyl-tRNAsynthetase	Translation	−3.2		

Adapted from Belfiore et al. [133]

6.2.2.2. *Lactobacillus curvatus CRL705*. Unlike proteomics, peptidomics has the potential to uncover processing sites of precursor proteins. These peptides should be analyzed in their native forms. However, database searching using non tryptic peptides is much less effective [158] due to the lack of charge localization at the N and C termini of the peptides. Also the poor fragmentation and the absence of sequence specificity is a problem for naturally occurring peptides [159]. In some cases, and has seen in the previous section, peptidomics has been advocated to the study of the meat peptides generated by the action of endogenous proteases some of them related to sensory attributes [160–164]. Even when muscle enzymes are greatly involved in meat protein degradation, bacterial proteolytic activity leads to a richer composition of small peptides and amino acids which contribute to the ripening process either as direct flavor enhancers or as precursors of other flavor compounds. A peptidomic analysis has been undertaken by Fadda el al. [165] to characterize the proteolytic compounds present in commercial fermented sausages. Results showed that low molecular mass peptides (LMW peptides) (between 1000 and 2100 Da) were arisen from both type of muscle proteins

indicating that myofibrillar and sarcoplasmic proteins were affected during fermentation and ripening. Due to the wide variety of cleavage sites deduced from their positions on the parental protein, the involvement of both muscle and bacterial proteolytic system on their production was suggested. Further in vitro studies, using a beaker sausage model, have been carried out to identify fragments of meat proteins generated in the presence of a selected LAB strain, *Lb. curvatus* CRL705 compared to an un-inoculated control treated with antibiotics. Results obtained from the LC-ESI-MS analyses showed a significant increase of LMW peptides after 10 days of incubation at 25 °C in both studied conditions (sterile and inoculated) due to the action of both muscle and bacterial proteolytic system.

However, the presence of the *Lb. curvatus* strain produced a different pattern of peptides when compared to the control. Meat proteins showed to have different hydrolysis susceptibility depending on the presence or absence of *Lb. curvatus* CRL705. For instance peptides fragments arisen from actin and myosin light chain 2 were registered only when the *Lb. curvatus* CRL705 was present. This could be due to bacterial peptidase and aminopeptidase activities as well as to the activation of some muscle enzymes under the fermentation conditions prevailing in the meat matrix. In fact, the acidogenic metabolism of *Lb. curvatus* during meat fermentation could promote the action of some proteolytic enzymes such as cathepsins (Lopez, C. et al. personal communication). This confirms previous works where the contribution of LAB to meat proteolysis during meat fermentation was proposed [166–168]. Tandem MS analyses enable us to obtain the sequence of the peptide as well as its position and cleavage site on the parental protein, this indicating to be an accurate method to characterize proteolytic metabolites from this kind of products. In addition, two dimensional electrophoresis studies are in progress to evaluate meat protein degradation under the same conditions but focusing on larger proteolytic products (100–10 kDa). Additional work is however still needed to further determine the potential of these peptides as flavor biomarkers for differentiating technologies or potentiating LMW peptide production to improve the flavor of fermented meat products.

6.2.2.3. Coagulase negative Staphylococcus: S. xylosus C2A. *Staphylococcus xylosus* is a saprophytic bacterium commonly found on skin of mammals but also used for its organoleptic properties in manufacturing of fermented meat products. This bacterium is able to form biofilms and to colonize biotic or abiotic surfaces. In multilayered biofilm, cells are embedded in an exopolysaccharide matrix. Biofilm formation process is mediated, to a certain extent, by cell-envelope proteins. A proteomic approach were carried out to characterize cell-envelope subproteome of the strain *S. xylosus* C2a [134]. The same strain was also studied to gain further insight into the physiology of biofilm formation following a comparative proteomic analysis between planktonic and sessile cells. Results showed differentially expressed proteins mainly associated with nitrogen and carbon metabolisms, essentially amino acid biosynthesis and glycolysis/TCA cycle, respectively. Besides, up-expression of several enzymes related to EPS biosynthesis in *S. xylosus* biofilm was revealed. Authors suggest that some secreted proteins could have a role in the formation of *S. xylosus* biofilm. In fact several primarily cytoplasmic proteins found on the bacterial cell surface might moonlight and have adhesive properties

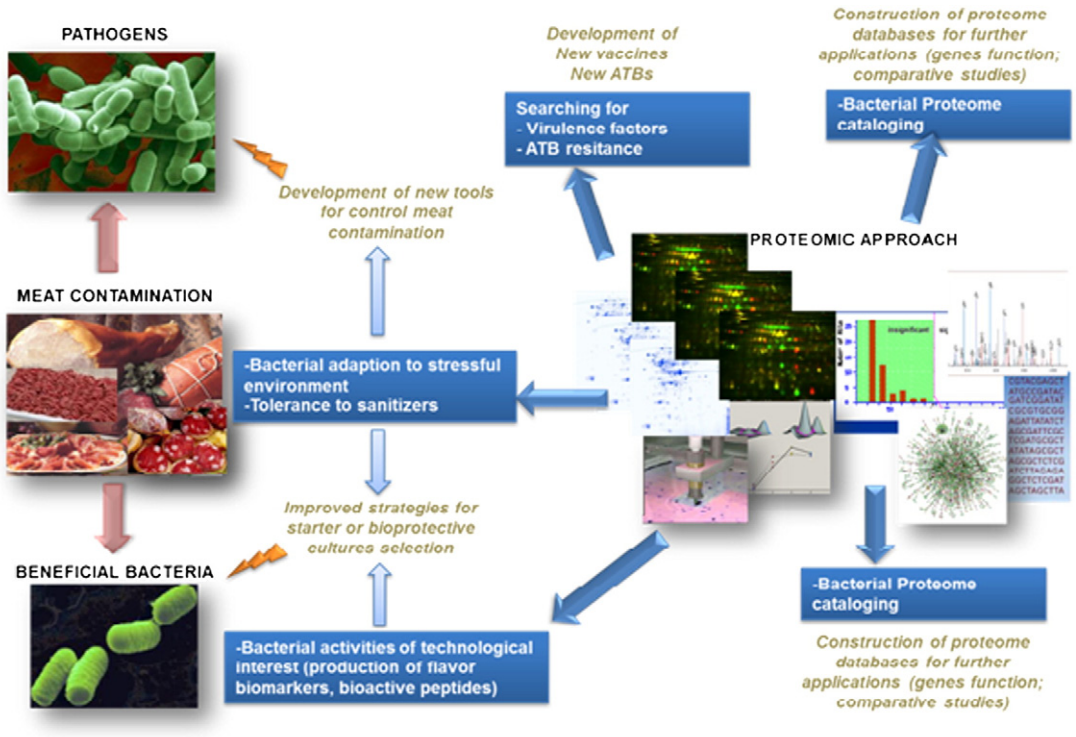


Fig. 3 – Schematic representation of the applications of proteomics in the context of research in meat and meat products microbiology.

playing a role in the formation of biofilm, such as enolase, showing plasminogen binding activity. The over expression of enzymes related to polyketide biosynthesis in *S. xylosus* biofilm, suggests that such pathway are possibly required for sedentary lifestyle. Then, how this polyketide pathway is related to *S. xylosus* biofilm formation remains to be elucidated [135].

6.3. *Proteomics, meat and microbial contamination—concluding remarks*

As previously discussed proteomics demonstrated to be a powerful tool to elucidate molecular mechanisms of bacterial physiology (see schematic representation in Fig. 3). Different objectives can be addressed such as studies of pathogen adaption to processing environments or the analysis of virulence factors and the regulatory network involved in pathogenesis. On the practical side there is the potential of developing novel targets for vaccines and therapeutic drugs. There remains, however, the technical limitation of determining the proteomes of the bacteria when they are growing in association with their host. On the other hand proteomics are also efficiently applied on the research of positive technological meat microbiota that will contribute to efficiently select the best starter cultures for meat fermentation and biopreservation. Also peptidomics showed to be efficient to identify peptides originated by bacterial enzymatic activities. There is little doubt that proteomics has provided us with new and valuable data and will continue to be an important source of information in the coming years.

7. General conclusions and future prospects

Meat and meat products are essential features in human nutrition, economy with strong cultural and affective implications in virtually all regions of the globe. Being, fundamentally proteinaceous products, it is vital to know the major protein-related events underlying all the different steps in the manufacture chain and holding an integrated viewpoint of the whole chain, the frequently called “farm to fork” perspective. As seen in this article, such proteomics studies are of vital importance not only to the characterization of the product, as well as their nutritional, economical and cultural values. So far, and despite its importance, proteomics studies in the field have been hindered by several factors. Chiefly, these include the little access, or even unawareness, of meat scientists to high-throughput, or standard proteomics methodologies that rely on costly instruments and highly skilled technical staff, frequently unaffordable to most meat science research institutes or available funding for the sector. It is vital therefore to increase the collaboration between meat scientists, proteomics platforms, as well as the industry itself to overcome such difficulties.

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